Laboratory and marine trials of resistance of furfurylated wood to marine borers

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ABSTRACT

New approaches are needed to prevent borer attack in wood intended for use in the marine environment, because traditional treatments are restricted or prohibited in this end use. Wood modification is a promising alternative, but the modification processes may require optimisation for marine end use. The standard testing method as defined by EN275 requires long term marine testing in which an iterative approach to process refinement is not feasible. This study used rapid laboratory testing protocols and marine tests to evaluate the efficacy of furfurylation in reducing attack by marine wood borers. Laboratory trials provided rapid assessment of potential treatments under controlled conditions, whilst marine trials provide more robust long term results under realistic exposure conditions. The wood samples were treated with one of three proprietary procedures for inducing polymerisation. Established methods for testing with the boring crustacean *Limnoria* allowed comparisons to be made with known timber species with a reputation for durability under marine conditions. Wood modification was shown to achieve useful reductions in feeding rate and survival of test organisms. Statistically robust results were obtained within four weeks. New methods for assessing the effects of wood modification on settlement and subsequent grown by shipworms yielded similarly robust and rapid results. The findings were compared with data for the same wood modifications obtained in a standard marine trial. Field and laboratory findings were in broad agreement. The laboratory methods were shown to be suitable for iterative optimisation of modification.

INTRODUCTION

Wooden structures in seawater can become rapidly degraded by attack from marine borers which use the wood as their food source and habitat. In Europe, the marine wood boring species that pose the most significant threat are from the bivalve mollusc family Teredinidae (shipworms) and the isopod crustacean family Limnoriidae (gribble) (Borges *et al.*, 2014 a & b). Both types first break wood down mechanically: shipworms with their ridged shells and gribble with their cutting and grinding mouthparts. The wood is then digested via a suite of enzymes, glycoside hydrolases and others, which enable the borers to depolymerise polysaccharides (King *et al*. 2010; O'Connor *et al.* 2014).

Preservation of wood using broad spectrum biocides such as creosote or chromated copper arsenate (CCA) has, until recently, provided economically-viable protection against borer attack. However, legislation, such as the EU directive affecting CCA treatment (European Commission, 2003), limits the use of these broad spectrum biocides in the marine environment. Tropical hardwood species that display natural durability to borer attack often come from unsustainable sources and logging is consequently restricted. The development of a non-toxic yet effective method of wood preservation is thus urgently needed. The chemical modification of wood as a means to inhibit biodegradation offers a promising solution since modification only affects organisms ingesting the wood. The effective use of furfurylation is well established (Amaury *et al*., 1988; Anaya *et al*., 1984; Goldstein and Dreher, 1960; Westin, 1996). In this process, molecules of furfuryl alcohol become polymerised within the cell walls resulting in the improvement of desirable properties of wood such as dimensional stability and hardness (Lande *et al.* 2004), which may also aid resistance to biodegradation. More recently, the modification method has been adapted (Westin 1996) and in tests, the resulting leachate produced demonstrate lower toxicity units (TU) when compared to restricted broad spectrum biocides such as CCA (Pilgard *et al.* 2010). In addition, modification of wood with furfuryl alcohol is an economically and environmentally attractive method since furfuryl alcohol can be derived from renewable biomass such as corncobs or sugar cane (Lande *et al.* 2008).
This project set out to assess the effectiveness of three furfuryl alcohol treatment processes as methods for increasing resistance to marine wood borers. The animals selected for laboratory assessment were those found to pose a widespread risk in European waters: the shipworm *Lyrodus pedicellatus* and the isopod *Limnoria quadripunctata* (Borges *et al*., 2014 a & b). Laboratory trials were used to measure durability in terms of reduction of feeding and increase of mortality rates of *L. quadripunctata*, and reduction of settlement capabilities of *L. pedicellatus* larvae.

EXPERIMENTAL

Timber from maple (*Acer platanoides)* and pine (*Pinus radiata)* was cut into planks. Part of each plank was treated and part untreated to provide within-plank control for each treated sample. The planks from each species were impregnated, cured and dried with schedules designed to control polymerisation withing the wood. Three treatment and polymerisation processes, designated in this paper as A, B and C were tested.

Laboratory feeding trial with Limnoria quadripunctata

Test sticks measuring 20 x 5 x 2 mm were produced to provide five replicates per plank; twenty five replicates of each treatment process/ wood species combination, giving a total of three hundred test sticks. The test sticks were placed into separate beakers (5 replicates per beaker) of 500 ml unfiltered seawater collected from Langstone Harbour. and leached for three weeks with a water change after two weeks. The sticks were then placed, one per well, into12 well cell culture dishes. The arrangement of the treatments and wood species within the dish was determined using a pseudo Latin square design to ensure that each sample was represented in each well position at least once. Five ml of seawater was pipetted into each of the cell wells and the cell culture dishes were placed into a light- and temperature-controlled growth room (20±2^{o}C) for four days prior to start of the experiment.

The specimens of *L.quadripunctata* used in the trials were cultured and maintained in balau (*Shorea* sp.) wood samples collected from the intertidal zone at Southsea, Portsmouth (Grid reference: SZ 638 984 GB). The wood and the animals were kept immersed in running seawater at Portsmouth Institute of Marine Sciences for approximately three months prior to the start of the experiment.
Three hundred animals were extracted from balau wood and incubated in a growth room at 20±2 ^{o}C in seawater collected from Langstone Harbour for a number three days prior to the onset of the experiment. Animals were then examined for the presence of rapidly beating pleopods (a sign of vitality), four puncta on the pleotelson (a characteristic of the species) and to ensure that none of the animals were gravid females. The selected animals were then placed, one per cell, into the cell culture dishes which were kept the growth room for a total of six weeks. The seawater was changed twice a week at which point the animals vitality and position on the wood was assessed.

Laboratory settlement trial with **Lyrodus pedicellatus** *larvae*

Six boards of wood from each treatment/wood species combination were leached in unfiltered sea water from Langstone Harbour. The boards were placed in an upright radial orientation at the bottom of cylindrical tank of seawater. A board of wood infested with *Lyrodus pedicellatus* actively releasing larvae was positioned within the tank for a period of 24 hours. Hemispherical pits created by larvae attempting to settle were counted under stereo microscope. Numbers of animals that had bored into and settled within the wood were recorded using X-rays.

Marine trial

Test boards measuring 25 x 75 x 200 mm were attached to frames in a ladder-like structure (Figure 1) and submerged at Olhão harbour, Portugal. After a period of six months the boards were removed from the harbour, fouling was detached and limnoriid attack visually assessed and rated according to EN 275 (1992). The boards were X-rayed in order to assess attack by teredinids using EN275 ratings (Figure 2). Boards that were deemed a ‘fail’ (rating 4) were replaced.

Figure 1: Boards of treated and untreated wood after submersion in Olhão harbour, Portugal for six months.

Figure 2: X-rays of boards of treated and untreated wood after submersion in Olhão Harbour, Portugal for six months. Shipworm attack is visible in panel 308(pine control).

RESULTS AND DISCUSSION

Laboratory feeding trial with Limnoria quadripunctata

Both treatment and wood type significantly affected faecal pellet production (Figure 3), but no effects specific to individual planks were detected. Animals fed on untreated maple and pine wood produced more pellets per day than those fed on modified wood, with pellet production significantly higher on pine than maple (GLM ANOVA with wood species and treatment as factors, Tukey's post hoc test). Animals fed on wood with treatment C produced significantly less pellets than those fed on wood with the other treatments. The feeding rates are comparable to those observed in previous trials that tested chemically-modified wood as a means to increase resistance to attack by limnoriids (Borges *et al.*, 2008) and in a some, demonstrate a higher resistance (Papadopolous *et al*., 2008).

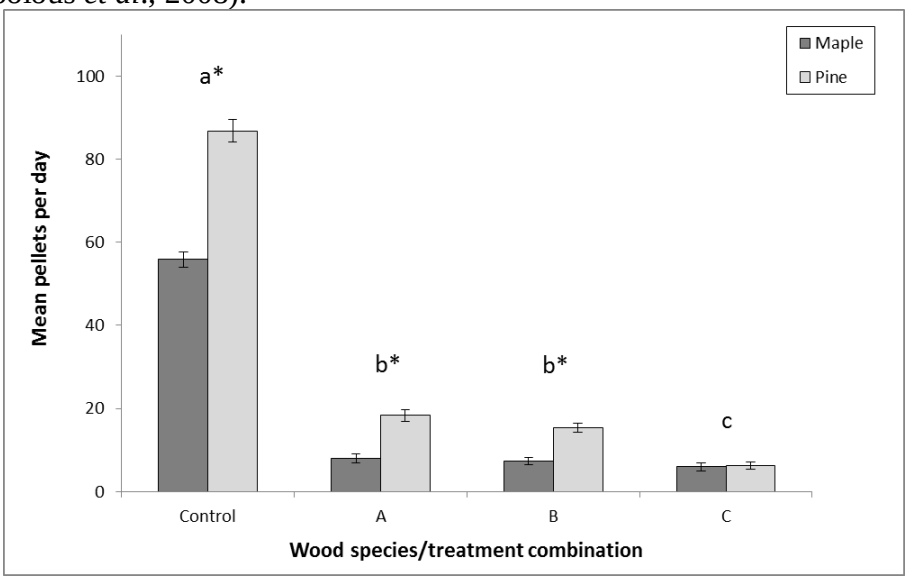

Figure 3: Effect of wood species and treatment on faecal pellet production by* L. quadripunctata *over a period of six week. (p<0.0005; Tukeys post hoc pair wise test of a GLM ANOVA model using treatment and wood species as fixed factors). Treatments that do not share a letter are significantly different. Asterisk indicates significant difference between wood types for a particular treatment.

Less than 10% of animals fed on control boards of either wood species died during this experiment, but mortality of animals fed on treated maple boards was more than 80%, whilst mortality of animals fed on treated pine boards with treatments A or B was 40-50% and with treatment C was over 80% (Fig. 4). Mortality on the treated wood was markedly higher than reported for animals fed on other wood with other modifications in previous trials (Papadopoulos *et al.*, 2007; Klüppel *et al.*, 2015) with comparable results to a trial with wood modified with DMDHEU and phosphobutane tricarboxylic acid (Borges *et al.*, 2004).

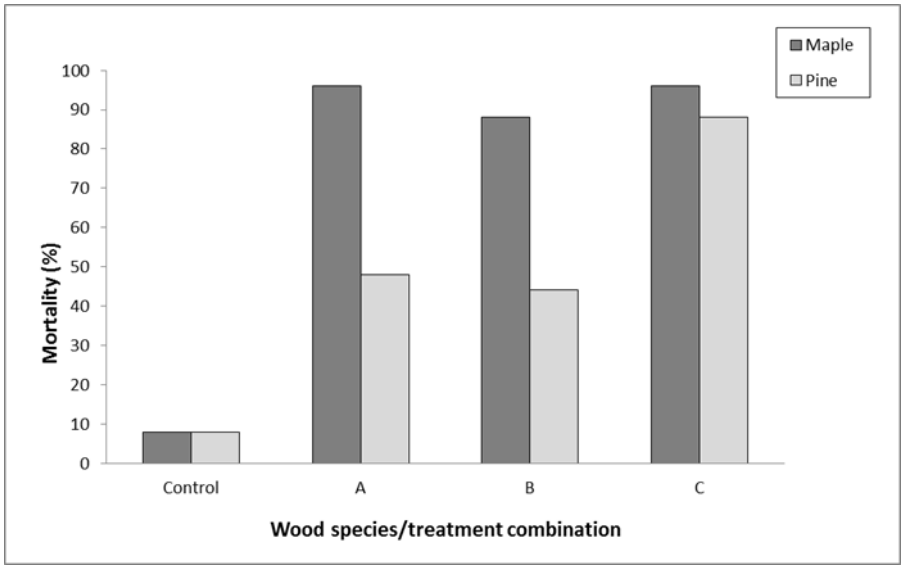

***Figure 4: Effect of wood species and treatment on mortality of* Limnoria quadripunctata**

Laboratory settlement and growth trial

The highest numbers of larval settlement attempts were observed on untreated pine wood whilst the least amount of attempts was observed on experimentally treated maple (Fig. 5). Overall, as with the feeding trials, treated maple wood was more resistant to settlement than treated pine wood. However, the experiment will need to be optimised and repeated as unexpectedly low numbers of live animals (<5) had successfully invaded the control wood samples and many dead juveniles were observed.

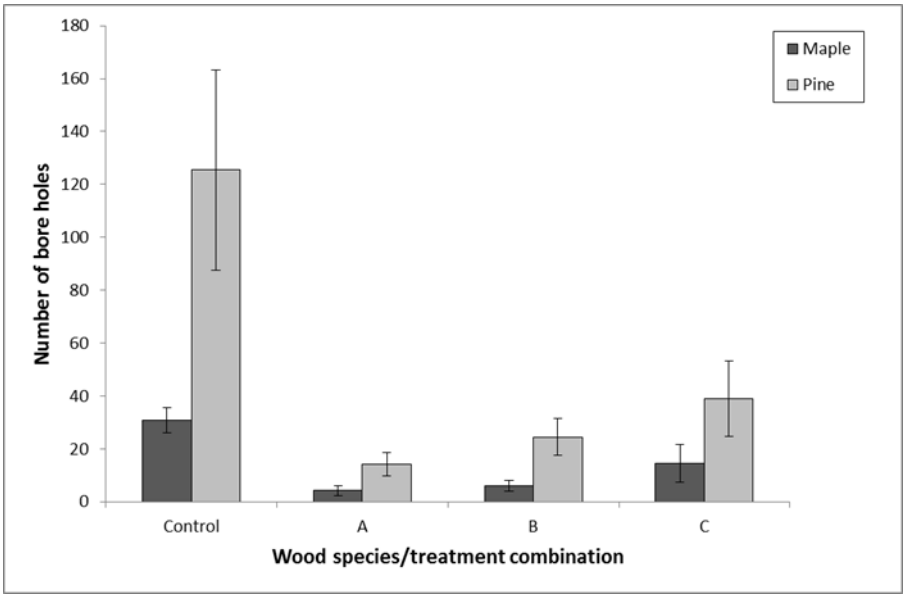

Figure 5: Effect of wood type and treatment on intensity of* Lyrodus pedicellatus *larval settlement

Marine trial

Untreated boards of maple and pine submerged at Olhão harbour Portugal failed after six months due to attack from both limnoriids and shipworm. However, both the naturally durable test wood azobé, and the boards of maple and pine treated with one of the three proprietary formulas either reduced or prevented attack (see Fig. 6).

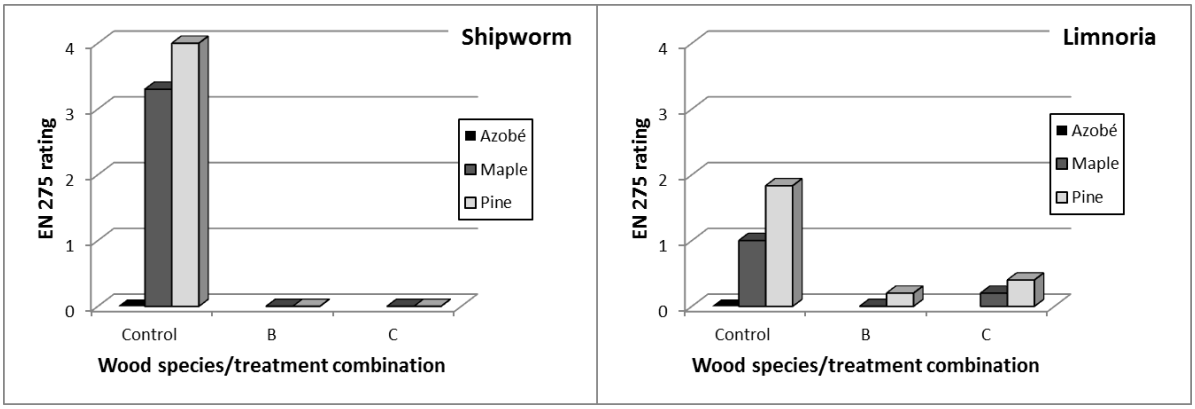

Figure 6: EN275 attack ratings of wood submerged at Olhão harbour after six months.

CONCLUSIONS

Furfurylation of maple and pine wood markedly reduced attack both by crustacean and by bivalve borers. In laboratory trials the treatments inhibited the ability of shipworm larvae to settle on and tunnel into the wood. The laboratory observations match initial findings in the marine trial. The settlement test with *L. pedicellatus*, however, requires optimisation to improve numbers of animals involved in the test and the condition of the wood tested. The marine trial needs to be continued to a duration of five years in order

to satisfy test standards set out in (EN275), but laboratory trials will permit optimisation of treatment methods over a much shorter period.

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